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'Proctoeces' sp. (Trematoda: Digenea) in the American Oyster, 'Crassostrea virginica'

(U.S.) Environmental Research Lab.
Gulf Breeze, FL

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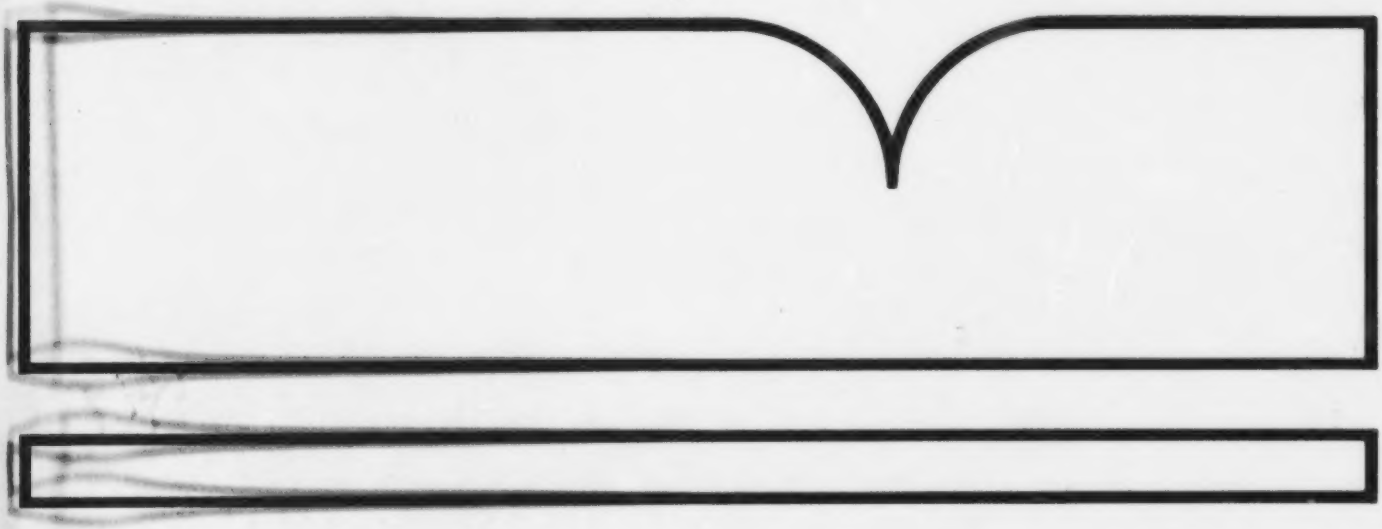

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16. ABSTRACT

Histological examination of over 6,000 oysters, Crassostrea virginica, inhabiting northern Gulf Coast estuaries revealed unencysted junvenile and possible adult stages of digenetic trematode, Proctoeces sp., inhabiting the gonadal ducts of the mollusc. The morphology of the worm is similar to juvenile stages of Proctoeces described in other bivalves. Infestation is highest in oysters from Mississippi Sound near Pascagoula, Mississippi with prevalence peaking in early winter and dropping off in spring and summer. The worm did not provoke a significant hemocytic response from the oyster. Oyster hemocytes were observed inside the digestive caeca of worms but there was no evidence of significant germinal feeding of gonadal impairment to the oyster. The hooked mussel Brachidontes recurvus, found attached to shells of oysters, may be significant in the life cycle of the digenean. The possibility of an altered life cycle in which the worm uses a surrogate host such as C. virginica in place of a vertebrate (fish) is hypotesized.

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Proctoeces sp. (Trematoda: Digenea) in the American Oyster, *Crassostrea virginica*[1]

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Abstract. Histological examination of over 6,600 individuals of the American oyster, *Crassostrea virginica*, inhabiting northern Gulf Coast estuaries revealed unencysted precocious metacercariae of a digenetic trematode, *Proctoeces* sp., infecting the gonadal ducts of the mollusc. Infection is highest in oysters from Mississippi Sound near Fascagoula, Mississippi with prevalence greater in later summer and early winter. The worm did not provoke a significant hemocytic response from the oyster. Oyster hemocytes were observed inside the digestive caeca of worms but there was no evidence of significant germinal feeding or gonadal impairment to the oyster. The hooked mussel, *Brachidontes recurvus*, found attached to shells of collected oysters, may serve as first intermediate host and thus be significant in supplying cercariae for the worms' opportunistic exploitation of *C. virginica* as a surrogate intermediate host or final host.

The Environmental Protection Agency[2] is currently studying the American oyster, *Crassostrea virginica* Gmelin, along the northern Gulf Coast to determine its potential as a bioindicator of pollutants in the estuarine environment. During the first 18 months of this study, histological examination of over 6,600 oysters revealed unencysted precocious metacercariae of a digenetic trematode, *Proctoeces* sp., inhabiting the gonadal ducts of this mollusc.

Although most digenetic trematodes become sexually mature in a vertebrate definitive host (Hyman, 1951), unencysted metacercariae, progenetic, and adult stages of the genus *Proctoeces* have been reported in prosobranch (Dollfus, 1964; Shimura, 1980) and lamellibranch molluscs (Freeman & Llewellyn, 1958; Stunkard & Uzmann, 1959; Wardle, 1980).

Presumed progenetic stages of *Proctoeces* spp. parasitizing bivalves in Japanese waters have been reported from *Ostrea gigas* by Fujita (1925) and *Brachidontes senhauisi* by Yamaguti (1938). Uzmann (1953) described unencysted progenetic larval stages of *Proctoeces*, (*Cercaria milfordensis*) from *Mytilus edulis* in tidal areas of Long Island and the Connecticut coast. He observed many well-developed eggs with motile miracidia in these worms and postulated the possibility that this trematode had a potential to ". . . abbreviate its life cycle . . ." by using *M. edulis* as a definitive host. Sporocyst and unencysted metacercariae of another fellodistomatid, *Cercaria brachidontis*, were reported by Hopkins (1954) coexisting in a mussel, *Brachidontes*

[1] The authors wish to thank Dr. Robin Overstreet of the Gulf Coast Research Laboratory in Ocean Springs, Mississippi and Joe Sypeck of the Virginia Institute of Marine Sciences for their help in the identification of the trematode.

[2] Part of a cooperative NCI-EPA Interagency Agreement on Environmental Carcinogenesis (Y01 CP 80205 Project 3).

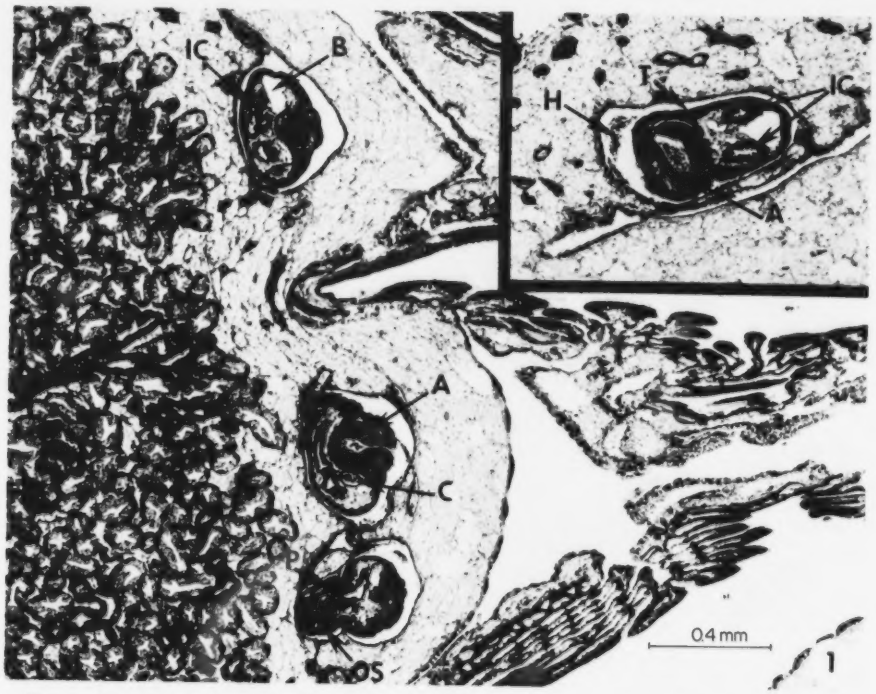

FIG. 1. *Proctoeces* sp. in gonadal ducts of *Crassostrea virginica*. Note absence of hemocytic response from oyster. T, tegument; OS, oral sucker; P, pharynx; IC, intestinal caeca; A, acetabulum; C, cirrus; B, bladder; H, hemocytes in duct.

recurvus, from Barataria Bay, Louisiana. Stunkard & Uzmann (1959) hypothesized that *C. brachidontis* and *C. milfordensis* were larvae of *Proctoeces maculatus*. Wardle (1980) described cercaria and unencysted metacercariae infesting the mussels *Ischadium recurvum* and *Mytilopsis leucopheata* from Galveston, Texas. He concluded that the worms he described also were larval stages of *P. maculatus* and conspecific with those described by Hopkins (1954), Uzmann (1953), and Stunkard & Uzmann (1959).

This paper reports a new bivalve host, *C. virginica*, harboring unencysted metacercariae of *Proctoeces* sp. and discusses aspects of this relationship.

MATERIALS AND METHODS

Three hundred oysters each month (100 per estuary) were collected from the Pensacola, Mobile, and Pascagoula estuaries. Sampling was initiated in August 1978 and data presented herein reflect histological examination of 6,600 oysters taken during the first 19 months of the investigation. The number of animals collected from Pascagoula was increased from 100 to 250 after 10 months.

TABLE I

Measurements of *Proctoeces* sp. from *Crassostrea virginica* and other bivalves (measurements in mm)

	Crassostrea virginica				*Ischadium recurvum*[2] (= *Brachidontes recurvus*)		*Mytilus edulis*[3]			
									Juveniles	
	Range	$\bar{x}$	N	50%[1]	Range	$\bar{x}$	Adult range	$\bar{x}$	$\bar{x}$ 2	$\bar{x}$ 1
Width	0.23–0.42	0.30	10	0.45	0.15–0.78	0.46	0.6–0.92	0.81	0.80	0.56
Acetabulum	0.17–0.25	0.21	10	0.31	0.24–0.35	0.30	0.35–0.46	0.39	0.35	0.21
Oral sucker width	0.10–0.19	0.14	10	0.21	0.17–0.24	0.21	0.21–0.32	0.27	0.19	0.12
Pharynx length	0.07–0.14	0.10	6	0.15	0.12–0.15	0.14	0.16–0.20	0.18	0.14	0.12
Testes	0.05–0.15	0.08	7	0.12	0.08–0.11	0.10	0.15–0.23	0.19	0.18	0.08

[1] Mean increased by 50% to allow for shrinkage due to fixation and compression.
[2] Wardle (1980).
[3] Stunkard & Uzmann (1959).

Transverse whole-body sections (2 cm) were taken from immediately behind the labial palps of oysters to obtain a representative area of mantle, gills, gonadal, and digestive organs. These tissues were fixed in Davidson's fixative (Shaw & Battle, 1957) and prepared for paraffin sectioning. Sections (7 μm) were stained with Harris' hematoxylin and eosin and examined at 25× and 50× with a standard microfiche reader (a method for examination of large numbers of oyster sections introduced by JAC) and when necessary, light microscopy. Since only histological sections were examined, measurements in Table I are approximate because of probable tissue shrinkage.

Results

Unencysted precocious metacercarial stages of *Proctoeces* sp. were found in the gonadal follicles and ducts of male and female oysters. Many oysters had multiple infestations of the trematode (Fig. 1).

General morphological features and dimensions of the sectioned trematode in the oyster are similar to *Proctoeces maculatus* described in *Mytilus edulis* by Stunkard & Uzmann (1959) and the mussels *Ischadium recurvum* and *Mytilopsis leucopheata* by Wardle (1980). Table I compares average measurements from worms found in *Crassostrea virginica* to those reported from other bivalves. Adjusted measurements that take into account histological tissue shrinkage and whole-mount compression are presented to obtain a realistic comparison with live specimens reported by others.

The trematode is cylindrical in shape and has an unarmed tegument which is strongly basophilic with hematoxylin (Fig. 1). A well-developed oral sucker extends into a muscular pharynx (Fig. 1). The width of the oral sucker is 0.14 mm (0.10–0.19 mm) and length of the pharynx is 0.10 mm (0.07–0.14 mm). Intestinal caeca are parallel and extend for 75% of body length (Fig. 1). A muscular, globose acetabulum 0.21 mm (0.17–0.25 mm) is located slightly

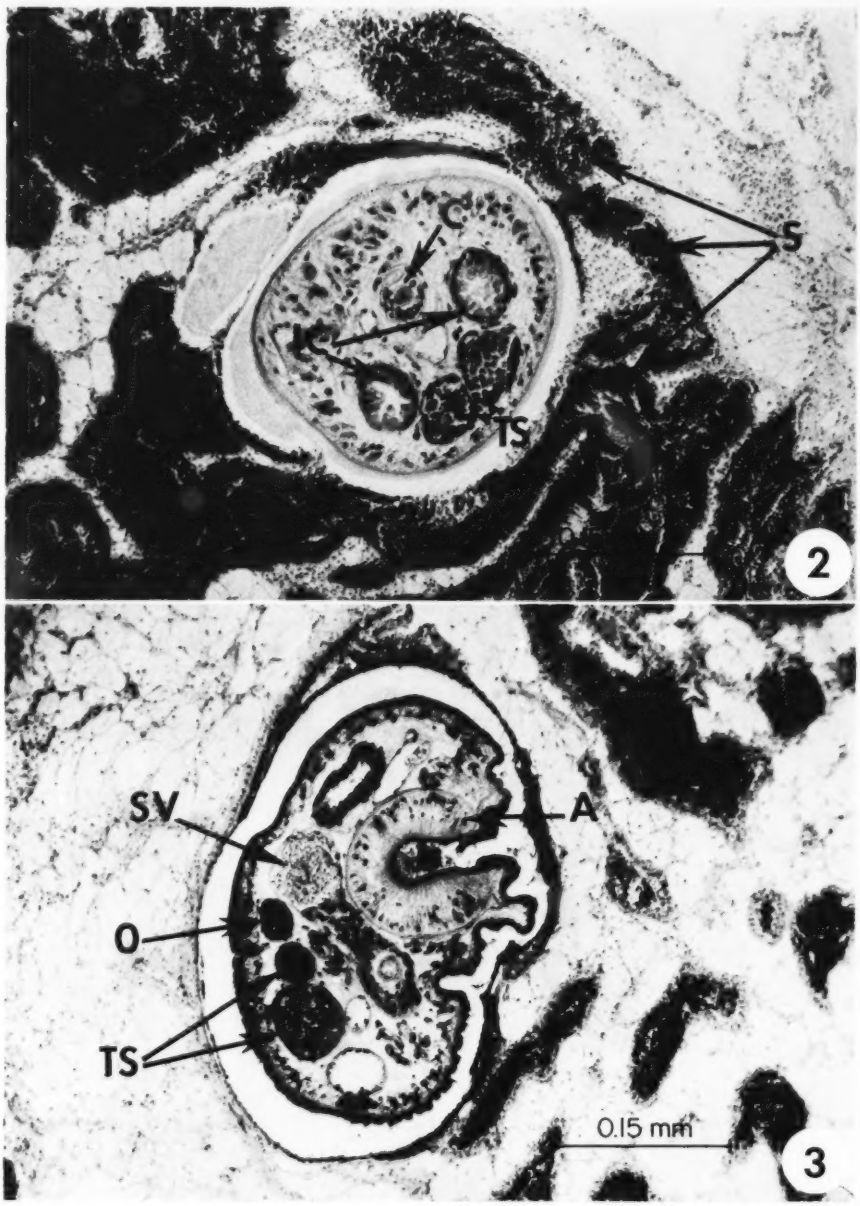

FIG. 2. *Proctoeces* sp. in gonadal follicle of *Crassostrea virginica* surrounded by sperm from oyster. TS, testes; C, cirrus; IC, intestinal caeca; S, sperm of oyster. Note sperm from oyster are not present in intestinal caeca of worm. FIG. 3. *Proctoeces* sp. in gonadal duct of *Crassostrea virginica*. TS, testes; SV, seminal vesicle; O, ovary; A, acetabulum. Note lack of hemocytic response in surrounding oyster vesicular connective tissue.

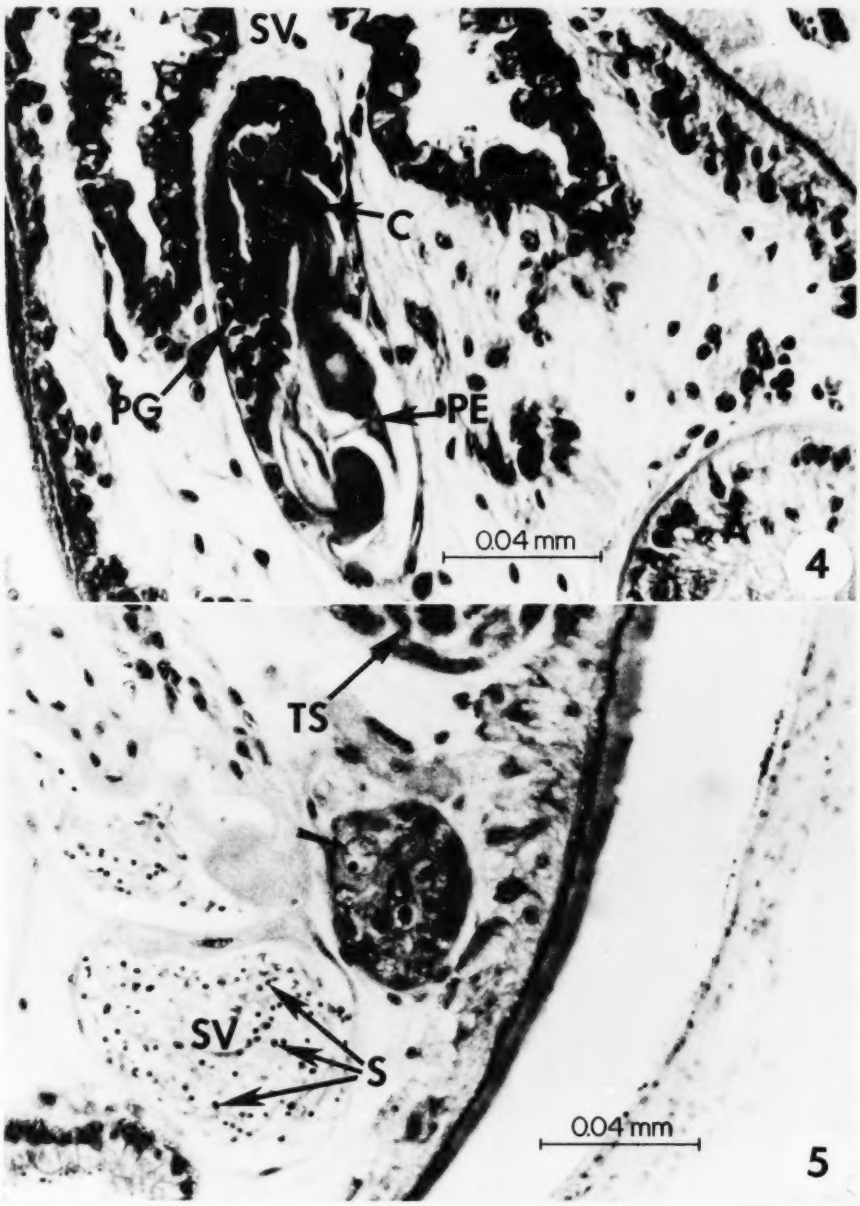

FIG. 4. Cirrus sac of *Proctoeces* sp. PG, prostate gland; PE, penis; C, cirrus; SV, seminal vesicle; A, acetabulum. FIG. 5. Ovary (O) from *Proctoeces* sp. with mature ova present (arrow). SV, seminal vesicle filled with sperm (S); TS, testes.

TABLE II

Prevalence of *Proctoeces* in *Crassostrea virginica* from the northern Gulf Coast

Estuary	Oysters examined	Oysters with *Proctoeces*	Prevalence %
Pascagoula	2,952	33	1.11
Mobile	1,796	5	0.28
Pensacola	1,879	10	0.53

anterior to the middle of the ventral surface (Figs. 1, 3). Developing and mature sperm are observed in the testes of many worms. The testes are connected by vasa deferentia to a cirrus sac located laterally to the acetabulum (Figs. 1, 3, 4). The cirrus sac consists of a seminal vesicle, prostate apparatus, and copulatory organ (cirrus-penis) (Figs. 3, 4). Many worms have seminal vesicles filled with sperm (Fig. 5). The cirrus protrudes into a canal or atrial duct which terminates at the genital pore and runs laterally and slightly anterior to the acetabulum. The female organs are not as well developed. No shelled ova are observed in females. When present in sections, a single ovary is located in the anterior part of the posterior one-half of the body between the acetabulum and testes (Figs. 3, 5).

Annual average prevalence of infestation was 1.11% in oysters examined

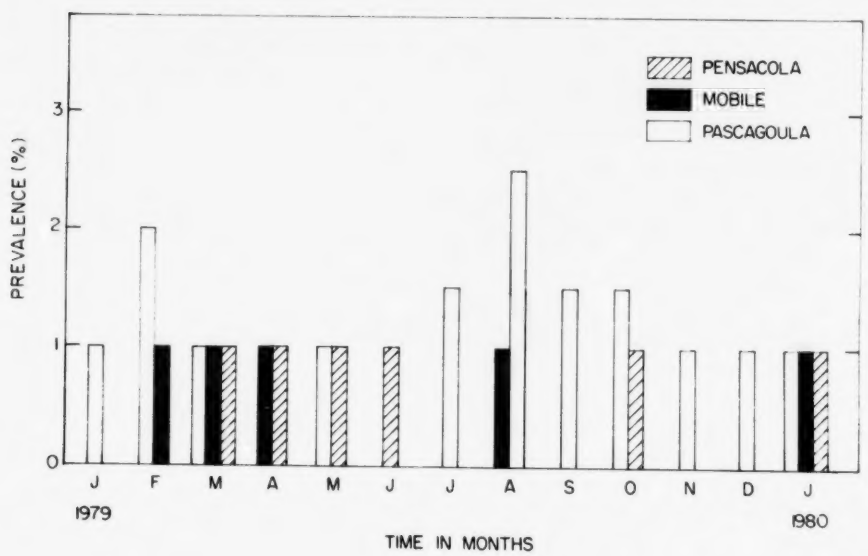

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FIG. 6. Annual average prevalence of infestation of *Crassostrea virginica* by *Proctoeces* sp. in Pascagoula, Pensacola and Mobile estuaries.

from Pascagoula, while Pensacola and Mobile oysters exhibited 0.53% and 0.28% prevalence, respectively (Table II). At Pascagoula, the trematodes were more numerous during the late summer (2.6%) and fall (1.3%) months (Fig. 6). Determination of a seasonal trend in Pensacola and Mobile could not be made due to the low numbers of worms found. The mussel *B. recurvus*, a known host of *P. maculatus*, was found attached abundantly to shells of oysters sampled from Pascagoula.

Oysters showed no significant hemocytic or other cellular reaction to the worm. However, hemocytes were seen inside the digestive caeca of many helminths, presumably from active feeding or accidental ingestion of oyster tissue or hemolymph. Although many worms observed were surrounded by oyster gametes (Figs. 2, 3), there was no evidence of significant germinal feeding by the worm or of gonadal impairment to the oyster.

Discussion

Although a whole mount of the trematode was not available, histological, morphological, and ecological characteristics indicate that the worm probably is a metacercarial or juvenile stage of *Proctoeces* sp. To the best of our knowledge, this is the first fellodistomatid reported from *Crassostrea virginica*.

Comparable morphological measurements show the worms from *C. virginica* to be similar to unencysted metacercarial and juvenile stages of *Proctoeces maculatus* from *Brachidontes recurvus* (Hopkins, 1954; Wardle, 1980), *Mytilus edulis* (Stunkard & Uzmann, 1959) and *Mytilopsis leucopheata* (Wardle, 1980). The worms in *C. virginica* varied in their developmental stages and although no shelled eggs were observed in the uteri of these worms, mature ova were observed; all worms had mature sperm present in testes, seminal vesicles, and cirri.

Although prevalence of *Proctoeces* sp. in *C. virginica* from Gulf Coast stations is lower than that reported for *M. edulis* (10.0%–30.0%) by Lang & Dennis (1976) and Tripp & Turner (1978), its seasonality is similar. The prevalence of worms in both molluscs is greater from late summer through early winter. Prevalence decreases from January through July until the late summer increase begins again. In *M. edulis*, Lang & Dennis (1976) reported that the increase of mature worms occurred after liberation of cercariae from mature sporocysts present in the mollusc. Once released, cercariae remained inside the host and developed into mature adults or escaped to infest another mussel. Since only unencysted worms were found in *C. virginica*, infestation can be assumed to be from a close external source.

A direct correlation between size of the mollusc and prevalence of the worm was not established for *C. virginica*. In the case of *M. edulis*, Lang & Dennis (1976) reported that larger clams were infested with more sporocysts but the number of mature worms was not dependent on size. A similar situation exists in *C. virginica* since the oysters found with worms were of varying sizes.

Tripp & Turner (1978) described a moderate hemocytic response in *M. edulis* to adult *P. maculatus*. *C. virginica* showed no hemocytic response.

The location of the worm in the oysters' gonadal follicles and ducts may limit significant hemocytic reaction since no vesicular connective tissue of the host is invaded.

The presence of oyster hemocytes in the worms' digestive systems suggests that the worms may be feeding on the oyster, or that accidental ingestion had occurred. Tripp & Turner (1978) observed hemocyte grazing by *P. maculatus* in the pericardial cavity of *M. edulis*; perhaps it is emulated in *C. virginica*. By irritating the host tissues with its oral or ventral sucker and initiating bleeding, a supply of food may become available. Evidence that the helminth feeds on the oyster's germ cells was not observed. Many worms were surrounded by germ cells of gravid oysters, but these cells were not seen inside the digestive caeca.

The greater prevalence of worms found in oysters from Pascagoula may be associated with the hooked mussel *B. recurvus*. A heavy attachment of this mussel was found on shells of oysters at this location. Sporocysts, cercariae, and progenetic stages of *P. maculatus* have been found in mussels from Louisiana (Hopkins, 1954), Mississippi (Overstreet, personal communication), and Texas estuaries (Wardle, 1980). This characteristic of the genus *Proctoeces* (i.e., to be found in different stages within a mussel) is well documented and discussed by Cheng (1967). The worm can mature and complete its life cycle within a single molluscan host. Stunkard & Uzmann (1959) and Lang & Dennis (1976) reported larval and mature adult stages of *P. maculatus* with embryonated eggs and active sperm in *M. edulis* from cooler New England waters. Serkova & Bychowsky (1940) and Stunkard (1959) were able to demonstrate experimentally the ability of trematodes to complete their life cycles using only molluscan hosts. Freeman (1962) proved that adult *Proctoeces subtenuis* from *Scorbicularia plana* could survive in experimentally infected fish. These data seem to support the postulate of Uzmann (1953) that *Proctoeces* has the capacity to abbreviate its life cycle by using a mollusc as a definitive host. A similar phenomenon may be occurring in *B. recurvus* with the added result of occasional transfer of cercariae to the oyster. Prevot (1965) postulated that the worms' developmental plasticity (in molluscs) appears to be greater in more temperate waters. The apparent lack of viable eggs in worms infecting oysters from warmer Gulf Coast waters, compared to reports of viable eggs in adult or progenetic worms (infecting molluscs) in more temperate New England and British waters, gives support to his hypothesis. A hypothetical exploitation of different hosts by *Proctoeces* sp. is presented in Fig. 7. It is probable that the digenean exits the mussel as a cercaria, becoming available to either a definitive fish host or another mussel, and contacting neither, enters the oyster's genital orifice by chance. From there, it penetrates a gonadal follicle or duct. The trematode m either mature and complete its life cycle using the bivalves as surrogate hosts or remain in a metacercarial stage awaiting a mollusc-eating fish (Sparidae or Labridae). Since no gravid worms with shelled eggs were found, the helminth probably remains as a young adult. However, its similarity with *S. plana* infections may indicate a possibility that complete maturity could occur if environmental conditions become selectively advantageous for such.

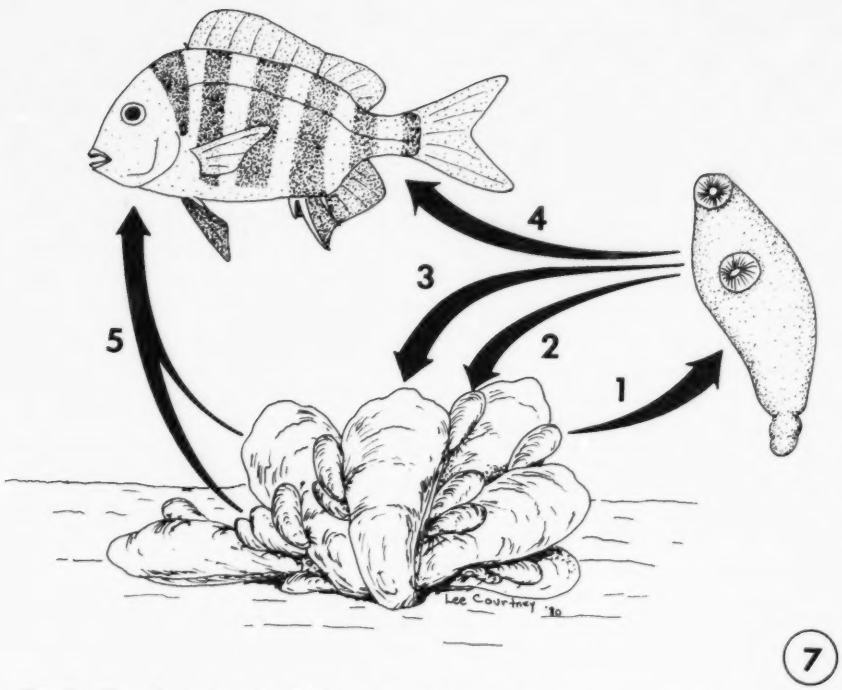

FIG. 7. Hypothetical modes of life cycle completion by *Proctoeces* sp. (1) Cercariae released from mussel may: (2) infest another mussel and mature; (3) infest nearby oysters and mature; (4) infest fish (sheepshead) and mature; or (5) fish either eats an oyster or mussel with a juvenile worm already present in one of the molluscs and the worm then matures in the fish.

Abundant *B. recurvus* might supply a large number of cercariae over time, many of which could infest oysters in the immediate vicinity of the generating mussel hosts. It is possible that the oyster acts as an intermediate host and maintains the worm in a presexual stage until eaten by a fish. Thus, another bivalve host, *C. virginica*, probably is being used by the trematode *Proctoeces* sp. We believe that the species which is infecting *C. virginica* as a surrogate intermediate host is *P. maculatus*. By using a known developmental plasticity common in this genus, the trematode is able to exploit several possible hosts for its continued survival.

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